日学術フォーラム「パンデミックに世界はどう立ち向かうのか」

# WHOにおける国際連携の最前線


## MOI MENG LING

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# COVID-19パンデミック以前

1. 多くはウイルス感染によって引き起こされる。
2. 治療薬、ワクチンが実用化されていない感染症が多く、5～15年周期で世界的に流行する傾向がある。
3. ヒトーヒト，動物ーヒト，節足動物ーヒトの感染経路で感染が成立する。

新興再興ウイルス感染症

| 疾 患 名 | 原因ウイルス（科） | 宿 主 | 分 布 | 治療法 |
|---|---|---|---|---|
| エボラ出血熱 | Ebola virus(filovirus) | 不明 | アフリカ中央部 | 対症療法 |
| デング熱 | Dengue virus(flavivirus) | ヒト | 熱帯・亜熱帯地域 | 対症療法 |
| マールブルグ病 | Marburg virus(filovirus) | 不明 | アフリカ中央部 | 対症療法 |
| ラッサ熱 | Lassa virus(arenavirus) | ネズミ | 西アフリカー帯 | リバビリン，免疫血清，対症療法 |
| クリミア・コンゴ出血熱 | Crimean-Congo hemorrhagic fever virus (bunyavirus) | 哺乳動物とダニ | アフリカ、東欧、中近東、中央アジア、インド亜大陸、中国西部 | リバビリン，対症療法 |
| SARS | SARS-CoV-1 | ヒト（哺乳類？） | - | 対症療法 |
| MERS | MERS-coronavirus | ヒト（ラクダ？） | 中東など | 対症療法 |
| ジカ熱 | Zika virus (flavivirus) | ヒト | 熱帯・亜熱帯地域 | 対症療法 |

など約20種類

# WHOにおける様々な感染症ネットワーク

## WHO-GOARNネットワーク

流行発生の状況把握、感染症対策（専門家派遣を含む）のためのWHOネットワーク

## WHO GLADS-HPネットワーク

世界に拡大するリスクのある高病原性病原体の診断、対策のためのWHOネットワーク

## WHO協力センター

WHOと協力し、地域カウンターパートの要請などに応じて実験診断、治療法、ワクチン対策などの活動に協力

## WHO専門家会議

実験診断、治療法、ワクチン、IDコントロールなど、必要に応じて立ち上がる場合もある

- 感染症を素早く検出し、早急に対策を実施
- ネットワークとの交流、キャパシティー強化

# WHO GLADS-HP ネットワーク

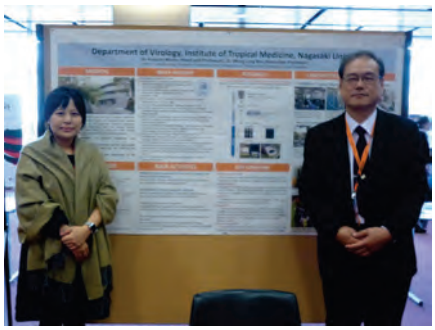
2019年 GLADS-HP専門家会議、ジュネーブ

新興・再興感染症においては、COVID-19の流行以前にもWHOを含めて、国際社会は強い危機感を持ち、“disease X”に対し技術・基礎研究基盤(new technologies)と迅速な対策実施 (rapid response)という2つのキーワードで戦略

(1) Rapid response towards emerging/re-emerging pathogens
(2) New technologies, rapid and reliable supply of reagents
(3) Strengthening global capacity
(4) Mechanisms to assure information and sample sharing during emergencies
>there are suggestions on possibilities of WHO to activate emergency sample sharing (as that of influenza network), pre-agreed legal instruments was also discussed
(5) Platforms for secure and fair data sharing during emergency

# WHO協力センター新興・熱帯ウイルス研究・レファレンスセンター

## 長崎大学熱帯医学研究所

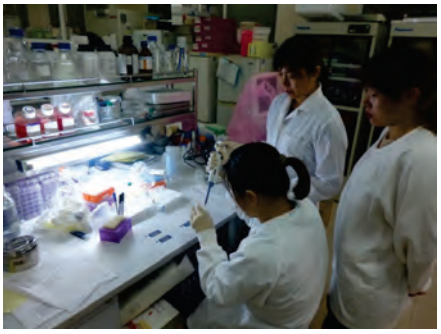


WHO GOARN focal point, WHO-CC Reference Lab for tropical viruses (JPN-67)

Comprehensive training for molecular diagnoses, SLMC Philippines


Pasteur Institute, Ho Chi Minh WHO training for advanced serology training for DENV& ZIKV differential diagnosis, Vietnam

WHO training for advanced serology training for ZIKV diagnosis, Hanoi, Vietnam



WHO training for advanced serology training for ZIKV diagnosis, Hanoi, Vietnam

Department of Medical Research, DMR eASIA project Myanmar-Japan-US

## Challenges in infectious disease research & control measures

**Rapid changes in needs**

**Need of innovative control measures**

**Need of support, technical & infrastructure**

# Zika virus associated microencephaly, Vietnam: 2015-


Cu Pong Village

西日本新聞 P.5

ジカ熱「小頭症と関連確認」

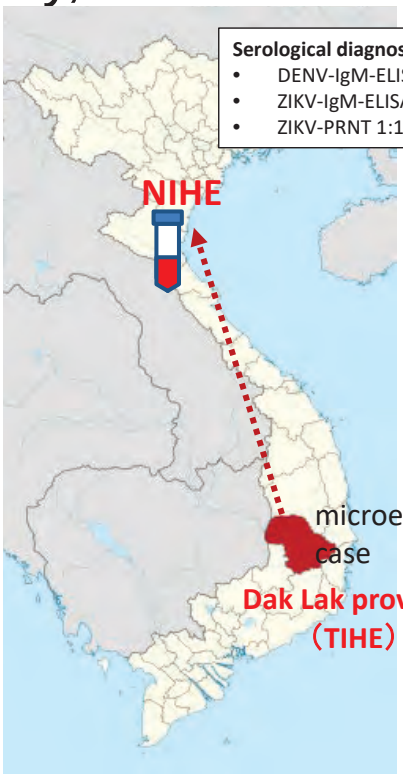
東南アで流行の感染症

長崎大などが研究成果発表

朝日新聞 2017年8月10日 朝刊 24ページ 東京本社

ベトナムの小頭症「ジカ熱原因」

Moi et al., Lancet Infect Dis., 2017




1. First case of microencephaly associated with ZIKV infection in Vietnam
2. Family members and neighbors were also positive for ZIKV infection but outbreak was limited within the village.
3. No further microencephaly case was observed beyond 2016 in Vietnam

# Sporadic Zika epidemic, Vietnam 2014-current

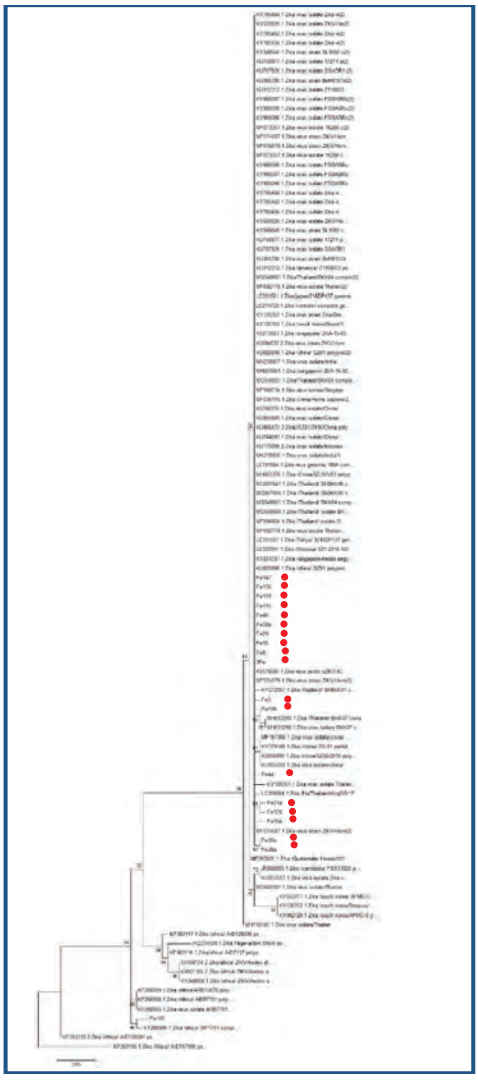
Nguyen Co Thach


- A total of 801 samples were collected from resident of Krongbuk district, Daklak province. By using the ZIKV IgM ELISA test, a total of 10.3 % of the serum samples (83/801) were positive for ZIKV IgM antibodies (P/N ratio = 3.30 ± 1.48), in which a majority of the positive cases correspond to children under age group of 15 years and the working age group 31-60 years
- Five of the nine samples (N=5/9) that demonstrated high levels of ZIKV antibodies was collected from the same village as that of the microcephaly case

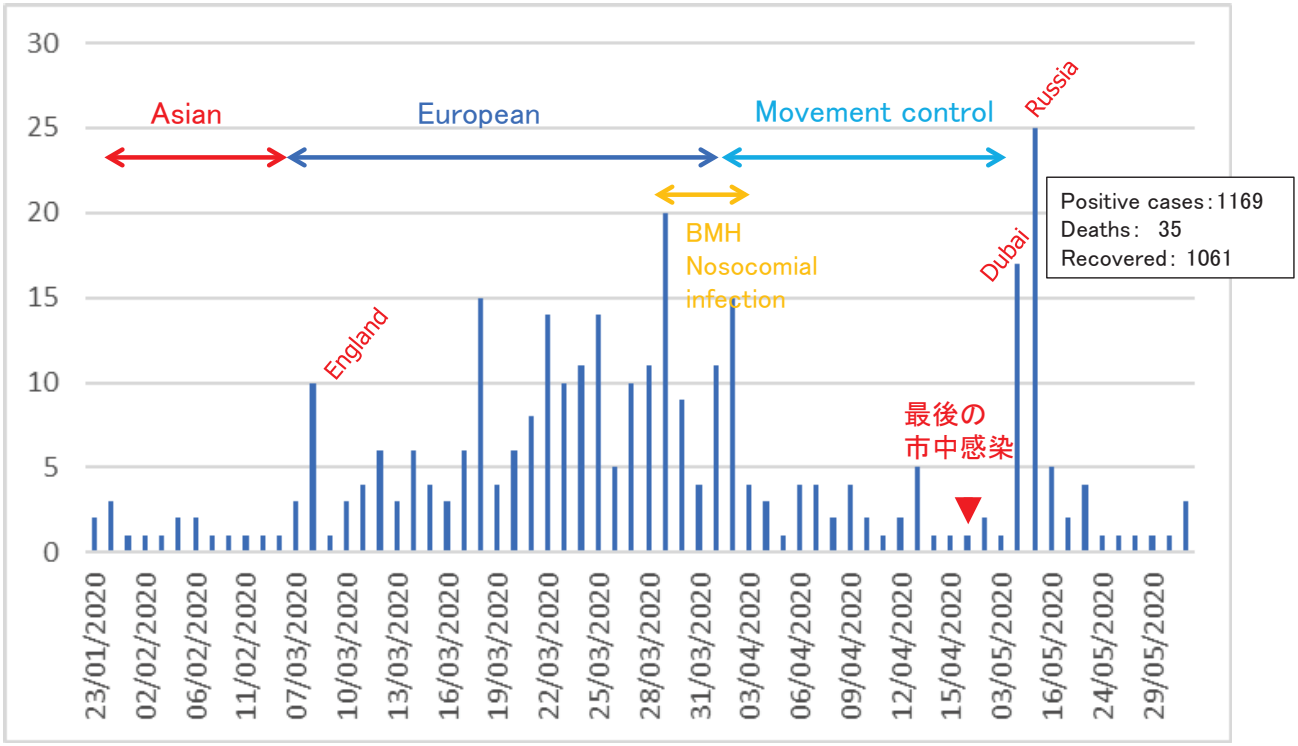
Lavangi W.

(1) A total of 599 serum samples collected from Hue, Vietnam and 180 serum samples collected from Hanoi, Vietnam in 2014-2015, were used in this study.
(2) Among 35 ZIKV positive samples, 16 samples (16/35 45.7%) were also positive for DENV IgM antibodies. Additionally, one sample (1/16, 6.25%) samples were positive for DENV NS1 antigen.
(3) NS5 region analyses reveals that the strains possess high homology with those of Asian (South American clade).
(4) These results suggest the occurrence of ZIKV in Central and North Vietnam prior to 2016.

Lavangi et al., 2020 Lancet Infect Dis., Nguyen et al., BMC Infect Dis 2020

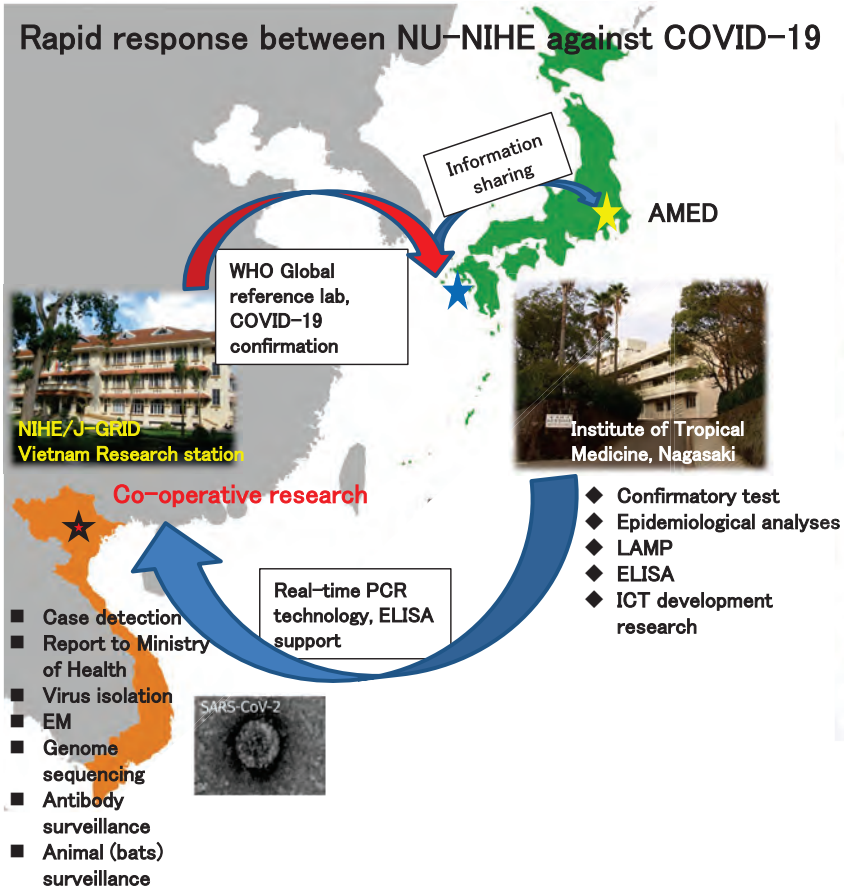


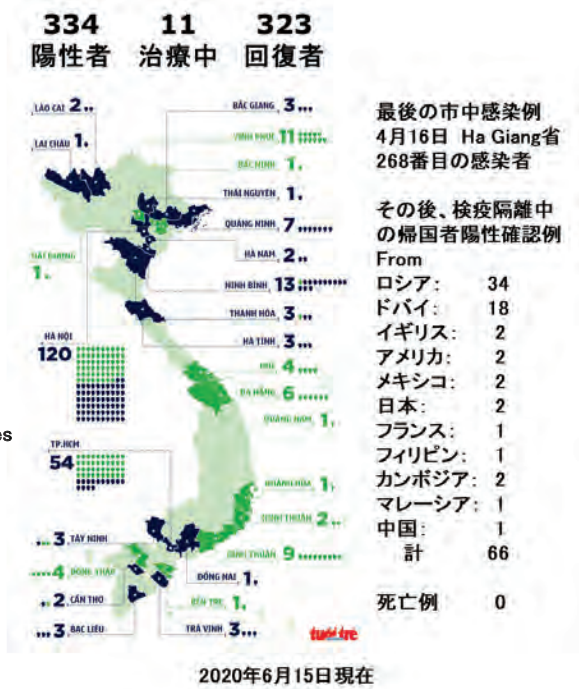
# COVID-19 situation in Vietnam




As of June, 2020.

# COVID-19 Response: NU-Vietnam Research Station




最後の市中感染例
4月16日 Ha Giang省
268番目の感染者

その後、検疫隔離中の帰国者陽性確認例

| From | |
|---|---|
| ロシア: | 34 |
| ドバイ: | 18 |
| イギリス: | 2 |
| アメリカ: | 2 |
| メキシコ: | 2 |
| 日本: | 2 |
| フランス: | 1 |
| フィリピン: | 1 |
| カンボジア: | 2 |
| マレーシア: | 1 |
| 中国: | 1 |
| 計 | 66 |

死亡例 0

# Our experience in collaboration with Vietnam on COVID-19

1. Since the inception of our Vietnam Research Station in 2004, in collaboration with NIHE, we have been conducting epidemiological studies of tropical and emerging virus diseases in the region.
2. Our collaboration includes research on Dengue outbreaks, Japanese encephalitis and Zika outbreaks.


3. In Jan 2020, there was a number of imported COVID-19 cases in Vietnam.
4. The first few cases of COVID-19 identified in Vietnam, was confirmed by NAAT/serological assays at Institute of Tropical Medicine in collaboration with NIHE.
5. There is a need of a highly specific and sensitive assay for COVID-19 differential diagnosis to other coronoviruses.

# Integration of new technology

### Technology review

(1) Upgrading diagnostic capacity
(serological: ELISA to PRNT;
virological: conventional Sanger sequencing to NGS),
adaptation from ZIKA PRNT
(2) Relevance of new diagnostic tools to current situation

↓

### Consultation

Internal and external
(WHOCC, WHO country office)

### Sustainability review

(1) Sourcing of materials
(2) Review of current available facilities
(3) Support for new technology

↓

### Consultation

Internal and external
(WHOCC, WHO country office)

### Training and support

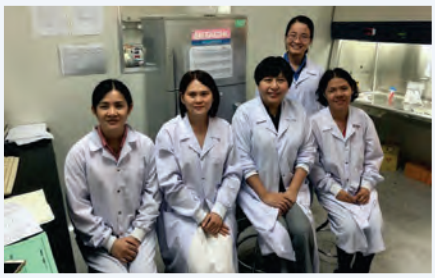
(1) Training of staff
(2) Technical support (results interpretation etc)
(3) Materials/reagents for quality control
(4) Evaluation of new technology

↓

### Improvement of infrastructure and integration to workflow


ZIKV serological diagnosis training, HINE, Hanoi, 2016

ZIKV serological diagnosis training, PI-HCMC, HCMC, 2017

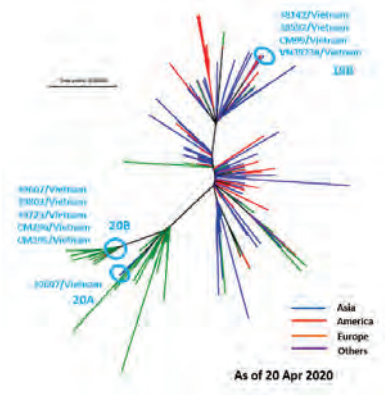


ZIKV serological diagnosis training, PI-NT, Nha Trang, 2018

Red indicates support from WHOCC (Japan side)

# SARS-CoV-2 Molecular studies: development of diagnostics (NU)

**Epidemiology : Phylogenetic Analysis of SARS-CoV-2 in Northern Vietnam**



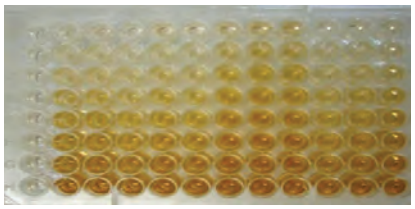

Three subgroups of SARS—CoV2 in Northern Vietnam

- 19B : Wuhan（Feb, 2020）
- 20B : European（March）
- 20A : European（March）

Le et al., EID, 2020;
Bastola et al., Lancet Infect Dis., 2020;
Ng et al., Int J Infect Dis., 2020.
Nabeshima et al., Lancet Reg Health, 2021

**Development of rapid diagnostics for SARS-CoV-2**

Rapid COVID-19 genome detection in cruise ship Costa Atlantica by using the LAMP method



Development of COVID-19 antigen/antibody detection assay

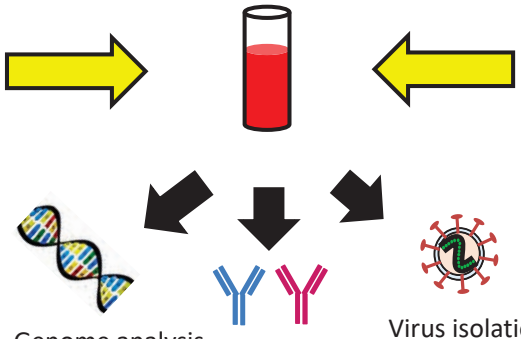
**International Collaboration**

- WHOCC, WHO global reference center for COVID−19 diagnosis: reagent and technology sharing

- Vaccine, therapeutics
- Basic research; pathogenesis

# SARS-CoV-2 surveillance in wild-life & natural reservoirs

Patients




Wild animals
Surveillance of bats in Vietnam for COVID-19


<u>Sampling from residents (patients) and wild-life。</u>

- SARS-CoV-2 by RT-LAMP developed in NU
- Virus genome analysis
- Virus isolation
- Antibody detection and neutralizing antibody titration
- Analysis of host factors（severe vs non-severe vs asymptomatic）

# What lessons were learned from this process?

| Incident | Lessons Learnt | Action Taken |
|---|---|---|
| (1) Increase the capacity for diagnosis of an emerging pathogen | (A) Need of new assays to confirm diagnosis results of conventional assays (ELISA, RT-PCR) | (A) Introduction of plaque reduction neutralization test (serology) and training<br>(B) Introduction and training of next-generation sequencing technology to increase diagnosis capacity<br>(C) Introduction of new technology after discussion with counterparts and evaluation of feasibility of plan with support from counterparts and WHO |
| (2) Identification and adaptation of suitable technology according to local needs and condition | (A) Materials and protocol that are optimized in WHOCC laboratory in Japan could not be sourced or has to be adapted to local conditions<br>(B) Need of control reagents and quality control measures | (A) Discuss with Vietnam counterparts, WHO country office to source suitable reagents<br>(B) Identification and optimization of locally available materials<br>(C) Provision of materials and reagents that are needed for the new assays<br>(D) Parallel performance of tests in WHOCC, NIHE and PI-HCMC |
| (3) Staff training & technical support | (A) Potential capacity gaps: laboratories may have differing levels of capacity in implementing the new technology<br>(B) Result interpretation: Different laboratories may have different interpretation of results (PRNT interpretation of primary/secondary flavivirus infection); NGS data interpretation | (A) Perform training courses in national laboratories in Vietnam in collaboration with WHO<br>(B) Discuss with Vietnam counterparts, WHO country office to better understand current situation and identify problems<br>(C) Continuous technical support, information and material sharing to ensure equal information sharing |

# How did the new technologies helped laboratories to better perform their functions?

| Improved functions | Description |
|---|---|
| Effectiveness | + The new assays has been implemented in diagnosis confirmation and epidemiological studies |
| Capacity | + National laboratory has improved capacity to perform advanced serological tests (PRNT) and virological tests (NGS) |
| Sustainability | + National laboratory is capable of performing the new tests in local settings |
| National implementation & technology sharing | + National laboratory staff are highly trained, possible introduction of technology to other national/province level laboratories<br>+ Possible application of new technology to further relevant research studies and control measures |
| Integration into overall public health system | + Possible integration of PRNT into national level diagnosis algorithm<br>(Adaptation of PRNT from the Zika diagnosis panel) |

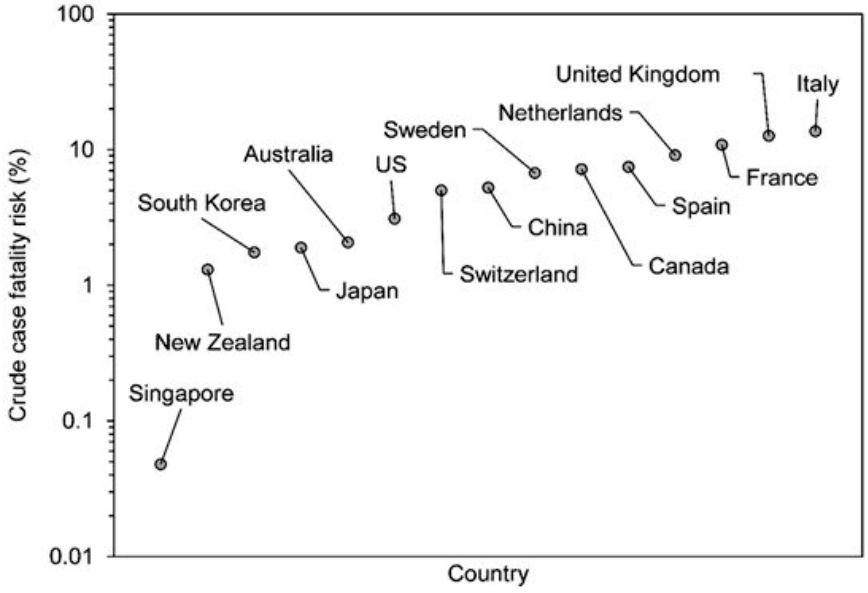
# 感染症対策のための研究開発

**(A)ウイルス分子疫学、病原体診断**
（1）ウイルス分子疫学
（2）病原体診断

（1）ウイルス分子疫学、ウイルス病態メカニズム解析
a. 長崎（日本）、世界的に流行するウイルス遺伝子配列解析
b. VOCの分離、in vitro/in vivoでの増殖解析

（2）病原体診断
a. N抗原ELISAの有用性検討
b. 遺伝子診断、プラークアッセイの評価
c. ADE抗体測定アッセイの構築

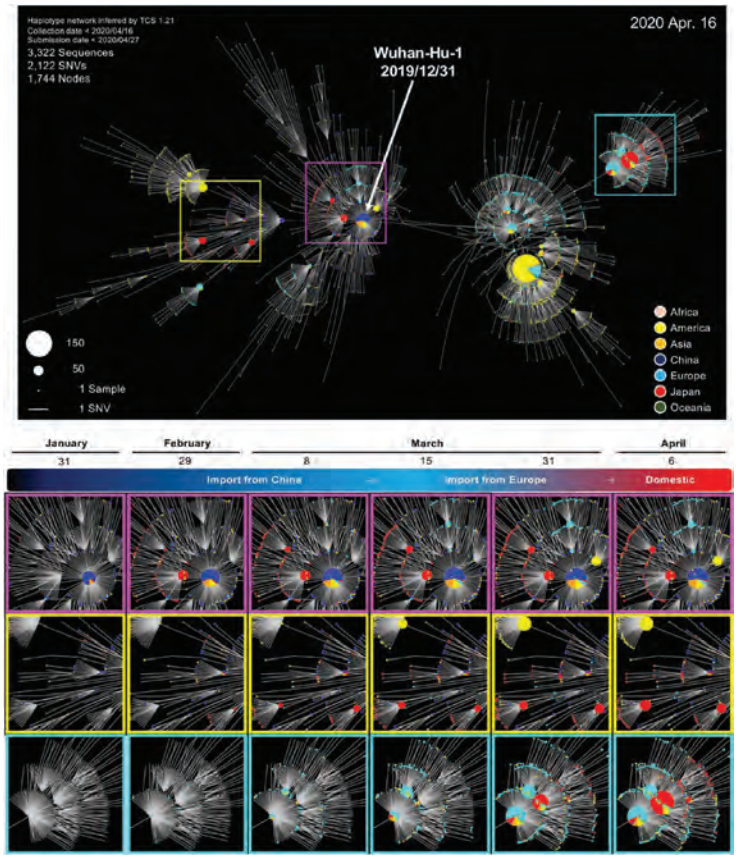
# COVID-19 case fatality in Japan




1. Overall COVID-19 CFR is relatively low for Japan, South Korea, New Zealand and Singapore is lower than that for Japan.
2. Low CFR estimate could be due to low-virulence SARS-CoV-2 variant, host factors and a high ascertainment rate owing to contact tracing.
3. New Zealand and Vietnam, used tough measures including closing borders, tight lockdowns, large-scale testing and strict quarantines - but Japan did not.

# Key question 1:
# Genetic changes & viral transmission/pathogenicity

**Haplotype network analysis using genome-wide single-nucleotide variations of worldwide SARS-CoV-2 isolates.**

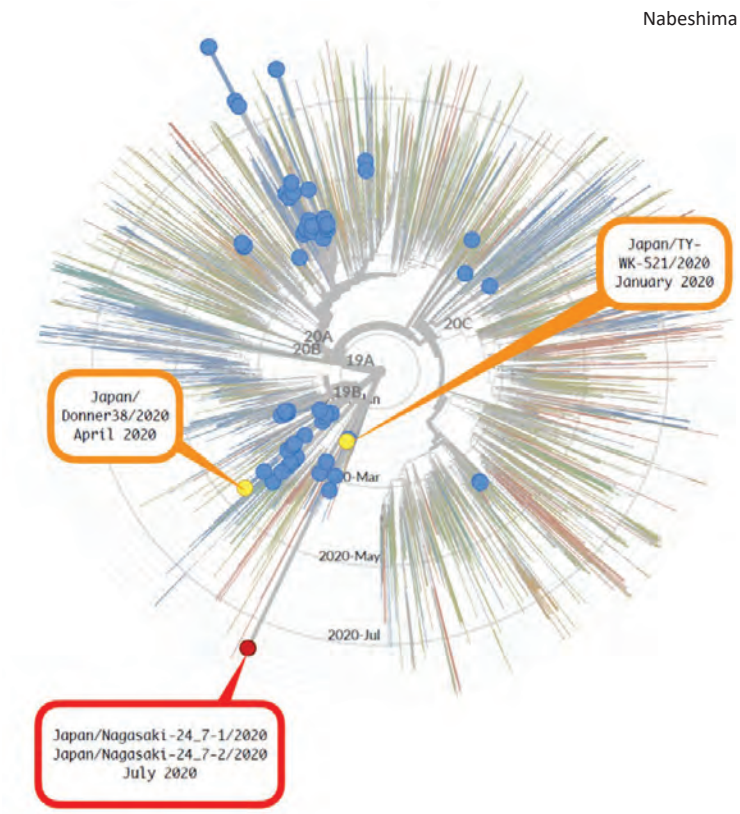



A) Whole-genome sequences of SARS-CoV-2 isolates in Japan (n = 435) were compared to all GISAID-available SARS-CoV-2 genomes (n = 2,887, updated on 16 April 2020) using TCS network analysis. SARS-CoV-2 disseminating from Wuhan City, China, at the end of December 2019 (one of the potential origins of Wuhan-Hu-1) is plotted at the centre of the haplotype network. B) Three plots of time-series cumulative COVID-19 cases were highlighted in each enclosed square to visualise the increasing incident COVID-19 cases.

Sekizuka et al., mSphere 2020

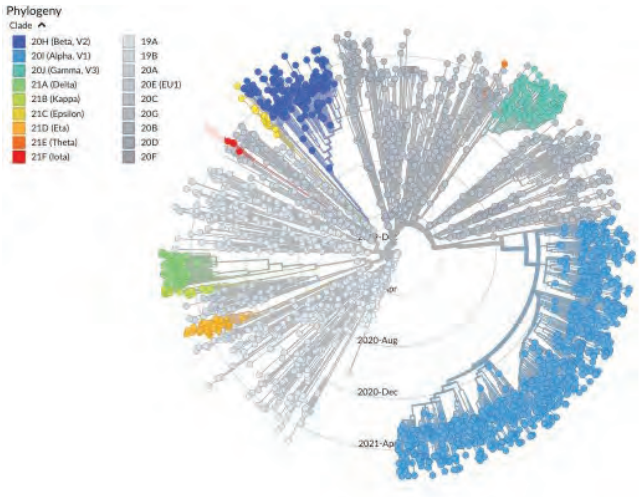
# Cryptic transmission of COVID-19 strains, Japan

Nabeshima et al., Lancet Global Health 2021

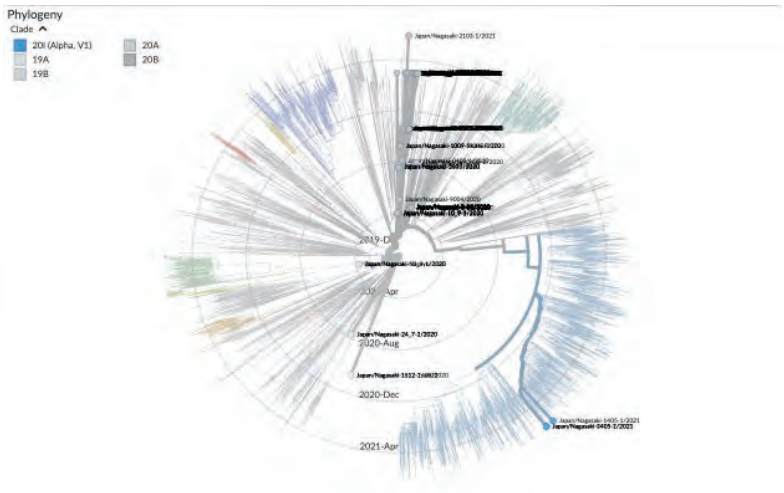



Maximum-likelihood phylogeny of 682 SARS-CoV-2 viruses collected from Japan (blue closed circles) on a background of 4981 globally collected virus strains from GISAID. Maximum-likelihood phylogeny of SARS-CoV-2 viruses collected from Japan (blue closed circles) on a background of 4981 globally collected virus strains from GISAID[3]. Nextstrain[5] was used to conduct phylogenetic analysis. Using Augur's subsampling process, 4981 records were selected from the GISAID records. The phylogenetic tree was constructed with the addition of strains isolated from Nagasaki. At the time point of September 2020, Nextstrain defined 5 major clades. Up to March 2020, the clades 19A and 19B (correspond to clades L+V and S in the classification of GISAID) were predominantly circulating in Asia. The clades 20A, 20B and 20C (G, GR and GH in GISAID) appeared in April 2020 and spread across Europe. In turn, the European clades became global dominant clade. The first case of COVID-19 was confirmed in January 2020 in Japan. Nagasaki prefecture is part of the Kyushu region of Japan and is located on the western edge of mainland Japan. The first case of COVID-19 in Nagasaki was confirmed in March 2020. Between March to April 2020, 16 cases were reported in Nagasaki prefecture, with no cases reported between May and June. Both strains of the clade 19B were isolated from patients in Nagasaki whom had returned from a neighboring prefecture.

# Transmission of COVID-19 strains, Nagasaki Japan (~2021)




2873系統全世界からの分離株




2873株中、長崎の分離株57株

# New variants summary

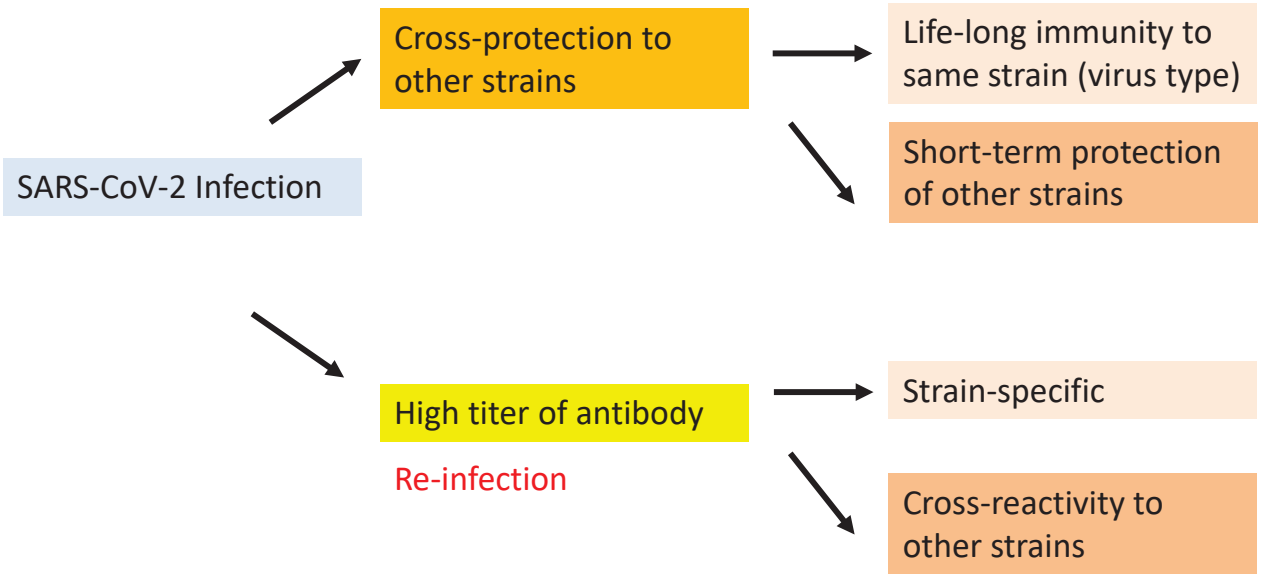
| Variant/ lineage | Reason for tracking | Cumulative UK sequences | Cumulative UK sequences (%) | UK Sequences over 28 days | UK Sequences over 28 days (%) |
|---|---|---|---|---|---|
| A.23.1 | International variant F157L, V367F, Q613H and P681R. | 162 | 0.06% | 17 | 0.04% |
| A.23.1 + E484K | UK | 62 | 0.02% | 6 | 0.02% |
| A.27 | France | 3 | 0.00% | 1 | 0.00% |
| B.1.1.318 | England | 1 | 0.00% | 1 | 0.00% |
| B.1.1.7 | UK associated variant. Has 17 mutations (14 replacements and 3 deletions) including: T1001I, A1708D, I2230T, SGF 3675-3677 del In the ORF1ab; 69-70 del, Y144 del, N501Y, A570D, P681H, T716I, S982A and D1118H in the Spike; Q27stop, R52I and Y73C in ORF8; D3L and S235F in the N501Y enhances ACE2 binding affinity P681H occurs at the furin cleavage site | 110732 | 40.48% | 36836 | 94.81% |
| B.1.1.7 + E484K | UK | 53 | 0.02% | 14 | 0.04% |
| B.1.1.7 + S494P | | 659 | 0.24% | 122 | 0.31% |
| B.1.351 | Variant associated with South Africa. Eight mutations in the Spike: D80A, D215G, E484K, N501Y, A701V, L18F, R246I and K417N. Three of these in the RBM, K417N, E484K and N501Y. K417N and E484K have been shown to escape some mAbs. | 270 | 0.10% | 69 | 0.18% |
| B.1.429 | California, USA. | 6 | 0.00% | 1 | 0.00% |
| B.1.525 | E484K, Q677H, F888L and a similar suite of deletions to B.1.1.7. | 83 | 0.03% | 38 | 0.10% |
| B.1.526 | New York, USA. | 2 | 0.00% | 0 | 0.00% |
| P.1 | Brazil. 10 mutations in Spike L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, H655Y and T1027I. | 5 | 0.00% | 5 | 0.01% |
| P.2 | Variant associated with Brazil. | 46 | 0.02% | 13 | 0.03% |

p://sars2.cvr.gla.ac.uk/cog-uk/

(PART B)

Key question 2: Host Immunity

# Research Question




**Research questions:**

1) immunity against SARS-CoV-2 contribute to protection or possibly pathogenesis
(2) different levels of immunity to circulating coronaviruses relates to SARS-CoV-2 susceptibility vs resistance,
(3) susceptibility to re-infection and
(4) isolation of potent and therapeutic neutralizing antibodies to SARS-CoV-2.

# Cross-reactive non-neutralizing antibodies in Dengue

**1. Development of an ADE assay**

Development of an ADE assay using FcγR-expressing BHK cells which is able to detect ADE activity of mAb and serum samples
Moi et al., *J Virol Methods*., 2010

**2. Determination of the utility of the ADE assay**

The ADE assay was useful in the determination of the sum of ADE and neutralizing activity as neutralizing antibody titer
Moi et al., *CVI*., 2010

**3. Determination of neutralizing antibody titers with the ADE assay**

(1) Discrepancy in the neutralizing antibody titer when determined by FcγR-expressing BHK cells
(2) Absence of neutralizing antibody to infecting DENV in patients
Moi et al., *PLoS NTD*., 2012

Findings:

| Activity to DENV | BHK | FcγR-BHK |
|---|---|---|
| Neutralizing activity | + | + |
| ADE activity | - | + |

PRNT assay that uses the FcγR-expressing cells may better reflect biological DENV neutralizing activity

Moi et al., Lancet 2012

# 基礎研究-ウイルス

**(A)ウイルス分子疫学、病原体診断**
（1）ウイルス分子疫学 (Lancet Glob Health., 2021など）
（2）病原体診断

（1）ウイルス分子疫学、ウイルス病態メカニズム解析
a. 長崎（日本）、世界的に流行するウイルス遺伝子配列解析
b. VOCの分離、in vitro/in vivoでの増殖解析

（2）病原体診断
a. N抗原ELISAの有用性検討 (Mutuantu et al., in prep)
b. 遺伝子診断、プラークアッセイの評価 (Mao et al., in prep, Fukuta et al., in prep)
c. ADE抗体測定アッセイの構築



# 基礎研究-免疫応答解析

**(B)免疫**
（1）エピトープ予測：ワクチン proof-of-concept
（2）防御免疫の誘導、持続免疫
（3）重症化リスク（HLA解析など）
（4）感染症流行予測

（1）エピトープ予測
a. ヒト・患者由来のBCR, TCR, HLA解析（開始予定）
b. エピトープ予測、in vitro/in vivoにて証明
c. 感染症ワクチンの解析基盤構築

（2）防御免疫・持続性
a. 後期患者のBCR, TCR, HLA解析、長期観察
b. 中和抗体と合わせたTCEMの頻度解析、共通エピトープ特定（開始予定）

（3）重症化リスク（RNAseq, metabolome, cytokine, HLA解析）
a. 重症・軽症コーホートの解析 (Fukuta, Matsumoto et al., in prep; LJI)

（4）感染症流行予測
a. TCEM解析、コロナ科交叉する免疫の解析
b. vitroで予測できなかったコウモリなどの人獣共通感染症はいままで、ヒトにどれくらいの頻度で感染しているか（接触頻度が高ければ人獣の壁を超える可能性も）
c. pre-COVID検体にてコロナ科、新興再興ウイルス感染症など、共通性の検討

# Final Message

1. Technologies
2. Policies
3. Social Integration
4. Collaboration

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